PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT	:	ARNTZEN, et al.
SERIAL NO	:	09/767,734
FILED	:	SEPTEMBER 29, 2000
TITLE	:	VACCINES EXPRESSED IN PLANTS
Grp./A.U.	:	1638
Examiner	:	BUI, PHUONG T.
Conf. No.	:	1914
Docket No.	:	P00245USD

DECLARATION OF DR. JOHN HOWARD UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Madam:

I, John Howard, hereby declare and state:

1. That I am the Chief Technical Officer of ProdiGene, Inc. the assignee of the above-identified application. Previously, I was Director of Biotechnology Research for Pioneer Hi-Bred International, Inc. for seven years, and Director of the Protein Products Division for two

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CERTIFICATE OF MAILING/TRANSMISSION (37 CFR 1.8(a))

I hereby certify that this correspondence is, on the date shown below, being:

MAILING
☐ deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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Date: 4/9/2003

LILA A.T. AKRAD

years. For nine years prior to this, I initiated and directed a biotechnology research program for crop improvement with Stauffer Chemical Company.

2. That I have a Ph.D. in Biochemistry from the University of California at Riverside. I have been involved in the science of the expression of proteins in plants for twenty years.

3. That I have read the office action dated December 9, 2002 and understand the Examiner is rejecting our claims regarding evidence of the generation of an immune response and that an animal upon exposure to transgenic plants transformed with viral antigens.

4. That in a study performed under my direction ("Delivery of subunit vaccines in maize seed", *Journal of Controlled Release* 85 (2002) 169-180), enclosed herein and described in detail hereinafter, we provided evidence in showing an antibody response in the animal fed a transgenic plant.

5. That two swine feeding trials were performed. The trial subjects were 10-12 day old seronegative piglets. The first trial was to examine serum from animals fed TGEV corn to determine they had produced neutralizing anti-TGE virus antibodies. The second study was designed to measure protection of piglets after a challenge with TGEV. In the first study, as outlined in the *Journal of Control Release*, piglets were divided into three groups. The animals were divided into three treatment groups. The control group received control corn meal; a second group was given TGEV corn meal, wherein the corn ratio was 50 grams of transgenic corn and 50 grams of wild-type corn. Piglets in the third group were maintained on normal rations throughout the course of the study. On day 29 all the animals were challenged with a 1 ml oral dose of virulent TGEV. Serum samples were analyzed for their ability to neutralize TGEV. Results of the neutralization titers are shown in Figure 4. Although neutralized antibodies were not detected in the serum of any piglets prior to virus exposure, administration of

whole virus resulted in a rapid induction of high levels of neutralized antibody in serum from piglets that had previously eaten TGEV corn. Therefore, a clear memory response leading to elevated levels of the neutralizing antibody was obtained and the animals fed transgenic corn containing recombinant TGEV-S antigens.

6. The second trial was designed to measure protection of piglets after a challenge with TGEV. Piglets were divided into five groups. One control group received control corn for 16 consecutive days; three groups were fed TGEV corn for either 4, 8, or 16 consecutive days, and one group received modified live virus vaccine as a positive control. The corn ration for each piglet was 50 grams wild-type corn or 50 grams transgenic corn (corresponding to approximately 2 mg of the S protein of TGEV). The piglets were then returned to regular water and medicated weaning rations. Piglets in the fifth group were orally vaccinated with the commercially available modified live TGEV vaccine on days 0 and 7, according to the label instructions. On day 18, all animals were orally challenged with a 2 ml dose of virulent TGEV. Following challenge, pigs were scored twice daily for signs of diarrhea and other symptoms such as dehydration and depression, anorexia, vomitus, moribund or death to give a total clinical score. To confirm viral challenge, fecal samples were collected from randomly selected animals within any group that produced watery diarrhea. These samples were tested for TGEV activity by inoculating confluent swine testicular (ST) cells in culture and staining by specific immunofluorescence.

7. That the percent morbidity incidence showed that 50% of the piglets fed wild-type corn developed TGE clinical symptoms (Fig. 5A). However, none of the piglets that received transgenic corn for 4 days exhibited symptoms.

8. That in another study performed under my direction ("Plant-based vaccines: unique advantages", *Vaccine* 19 (2001) 2742-2748), enclosed herein and described in detail hereinafter we also showed that animals fed TGEV corn could induce protection from a subsequent challenge using TGE whole virus, suggesting that feeding animals TGEV could result in generation of a protective immune response.

9. That the trial subjects were 10 day-old pathogen free piglets that were TGEV seronegative. Piglets were divided into three groups. The corn ration for one group consisted of either: (a) 100 grams of wild type corn or; (b) 50 grams of transgenic corn (corresponding to approximately 2 milligrams of the S protein of TGEV), mixed with 50 grams of wild-type corn; or (c) piglets orally vaccinated with the current commercially available modified live vaccine MLB-TGEV on day 0 and 7 of the study according to label directions. The piglets were then returned to regular water and medicated weaning rations.

10. That on day 12 all animals were orally challenged with a 2 ml dose of virulent TGEV. Prior work has shown that this dose should produce a clinically typical TGEV water diarrhea in 21 to 28 day old piglets that would persist for 7 to 10 days, but would not be lethal.

11. That following challenge, pigs were scored twice daily for signs of diarrhea and other symptoms of such as dehydration and depression, anorexia, vomitus moribund or death to give a total clinical score.

12. That to confirm viral challenge, fecal samples were collected from randomly selected animals within any group that produced watery diarrhea. The samples were checked for TGEV activity by inoculating confluent ST cells and staining by specific immuno fluorescence.

13. That the results showed all the piglets fed only wild-type corn developed TGEV clinical symptoms as shown in Figure 2A. By comparison, only 50% of those that received the

transgenic corn expressing the S protein exhibited symptoms. While 78% of piglets receiving a commercially modified live vaccine developed symptoms, indicating the transgenic corn vaccine was more effective in protecting the piglets.

14. That in a another study performed by Oragen Technologies & Veterinary Resources, Inc., on my behalf, described in detail hereinafter to determine if TGEV protein, corn-expressed vaccine can boost lactogenic immunity in animals that have previously been orally sensitized.

15. That in the study blood samples were collected at pre-breeding, 35 days prior to farrowing, day 14 and day of farrowing. Samples (serum, colostrums, and milk) were assayed for neutralizing titers using a varying antibody-constant virus assay.

16. That all animals were sensitized to TGEV and then boosted prior to farrowing. All animals were seronegative for TGEV at breeding. The animals were sensitized to TGEV by vaccination with a modified live TGEV vaccine. One group, Group G, did not receive any modified live TGEV vaccine and only received TGE transgenic corn throughout the study.

17. That the data showed that the TGEV transgenic corn was as effective as modified live TGEV in boosting the lactogenic immunity in animals that had been sensitized to TGEV. See as evidence the enclosed graph, "Sow Serum Response".

18. That the animals given only the transgenic TGEV corn were not sensitized to TGEV based on lack of neutralizing antibodies in serum (not shown in graph), colostrums, and milk of the vaccinated animals However, the absence of a measurable response does not indicate that the sows did not respond. When challenged (data not shown), the sows did exhibit a measurable antibody response, known as memory response. This "memory response" was also shown in our study described above published in *Journal of Controlled Release* entitled "Delivery of Subunit Vaccines in Maize Seed" (See Fig. 4) where piglets did not show a primary response, but when

subsequently exposed to the virus, responded with a significant serum response to an extent much greater than the control group.

19. That the above-identified studies demonstrate clearly that Applicant's invention would work at the time of filing as claimed in the above-identified patent application.

20. That we have shown evidence of a generation of an immune response in an animal upon exposure to a transgenic plant transformed with a viral antigen.

21. That the undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Apr 8, 2003
Date

John A. Howard, Ph.D.